

**Data Evaluation Report on the Acute Toxicity of DPX-MAT28 Technical
(Aminocyclopyrachlor Acid) to Fish (*Lepomis macrochirus*)**

PMRA Submission Number {.....}

EPA MRID Number 47560124

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D358148
	OECD Data Point	{.....}
	EPA MRID	47560124
	EPA Guideline	850.1075

Test material: DPX-MAT28 Technical **Purity:** 92.2%
Common name: Aminocyclopyrachlor acid Technical
Chemical name: IUPAC 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
CAS name 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
6-Amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylic acid
CAS No. 858956-08-8
Synonyms DPX-MAT28, Aminocyclopyrachlor

Primary Reviewer: John Marton
Staff Scientist, Cambridge Environmental, Inc.

Signature:
Date: 07/01/09

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Date: 07/20/09

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EPA/OPP/EFED/ERB1

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Date: 10/06/09

Reference/Submission No.: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
Use Site Category: {.....} [For PMRA]
EPA PC Code None

Date Evaluation Completed: 10/06/09

CITATION: Gallagher, S.P., T.Z. Kendall and H.O. Krueger. 2007. DPX-MAT28 Technical: A 96-Hour Static Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory report number 112A-214. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, DE. Study completed November 29, 2007.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.



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EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, bluegill sunfish (*Lepomis macrochirus*) were exposed to DPX-MAT28 (Aminocyclopyrachlor Acid) Technical at nominal concentrations of 0 (negative control), 7.5, 15, 30, 60 and 120 mg ai/L under static conditions; mean-measured concentrations were <0.0143 (<LOD; control), 7.5, 15, 29, 60 and 120 mg ai/L. The 96-h LC₅₀ was >120 mg ai/L. The EC₅₀ and NOAEC values were >120 and 120 mg ai/L, respectively, based on a complete lack of mortality and sub-lethal effects. Based on the results of this study, DPX-MAT28 Technical would be classified as practically non-toxic to *Lepomis macrochirus* in accordance with the classification system of the U.S. EPA.

This toxicity study is scientifically sound and classified as acceptable. It satisfies the guideline requirement for an acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): mean wet weight- 0.09 (0.05-0.13) g; mean total length- 2.1 (1.9-2.3) cm

Test Type (Flow-through, Static, Static Renewal): Static

LC₅₀: >120 mg ai/L 95% C.I.: N/A

NOAEC: 120 mg ai/L Probit Slope: N/A

EC₅₀: >120 mg ai/L 95% C.I.: N/A

Endpoint(s) Affected: None

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

This study was conducted following guidelines outlined in the OECD Guideline for Testing of Chemicals, 203, *Fish Acute Toxicity Test*; and U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines, OPPTS Number 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine* (draft). The following deviations from OPPTS 850.1075 were noted:

1. The TOC and particulate matter of the dilution water were not reported.
2. While one replicate of ten fish per test level is acceptable, guidelines indicate a preference of two replicates per treatment with 10 fish each to improve statistical power by reducing variability unrelated to treatment.

These deviations does not affect the scientific soundness or acceptability of the study.

COMPLIANCE:

Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Parts 160 and 792) that are consistent with: OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999), with the exception that the periodic analyses of well water for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test material DPX-MAT28 Technical (Aminocyclopyrachlor)

Description: Solid

Lot No./Batch No. : Not Provided

Purity: 92.2%

Stability of compound under test conditions: Stable. Mean-measured concentrations ranged from 96.7 to 100% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at ambient temperature.

Physicochemical properties of DPX-MAT28 Technical.

Parameter	Values	Comments
Water solubility at 20EC	Not Reported	

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Parameter	Values	Comments
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: Bluegill Sunfish (*Lepomis macrochirus*) EPA recommends a cold water species (preferably rainbow trout *Oncorhynchus mykiss*) and a warm water species (preferably bluegill sunfish *Lepomis macrochirus*). OECD recommends choice of species at discretion of testing laboratory.

Age at test initiation: Juvenile

Weight at study initiation: mean wet weight- 0.09 (0.05-0.13) g EPA recommends: mean 0.5 - 5 g.

Length at study initiation: mean total length- 2.1 (1.9-2.3) cm EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∇ 1.0 cm for bluegill and 5.0 ∇ 1.0 cm for rainbow trout

Source: Osage Catfisheries, Inc., Osage Beach, Missouri
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The nominal concentrations were selected based upon exploratory range-finding results and in consultation with the study sponsor; no details pertaining to the range-finding trial was provided.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u>		
Period:	At least 14 days	The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and $\leq 10\%$ = continued acclimation for 7 days; <5% = acceptable.
Conditions: (same as test or not)	Same as test	
Feeding:	Fish were fed daily with a commercially-prepared diet (Ziegler Brothers, Inc., Gardners, Pennsylvania); fish were not fed for at least two days prior to testing.	
Health: (any mortality observed)	No signs of stress were observed 14 days prior to testing and no mortality occurred during the 7 days prior to testing.	

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Parameter	Details	Remarks
		Criteria
Duration of the test	96 hours	<i>The recommended test duration is 96 hours.</i>
<u>Test condition</u>		
Static/flow-through	Static	<i>A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.</i>
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	N/A	
Aeration, if any	None reported	<i>Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.</i>
<u>Test vessel</u>		
Material: (glass/stainless steel)	Stainless steel	<i>Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm. Fill volume is usually 15-30 L of solution.</i>
Size:	54 L	
Fill volume:	40 L	
Source of dilution water Quality:	Water was obtained from an on-site well. Water was passed through a sand filter, pumped into a storage tank and aerated. Prior to use, water was filtered to 0.45 µm to remove fine particles.	<i>Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.</i>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u> Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	138 mg/L as CaCO ₃ (0-hrs) 7.8-8.5 ≥7.4 mg/L (≥85% saturation) Not Reported Not Reported See Reviewer's Comments None Detected 4.8 mg/L (Chloride) 21.5-22.8°C N/A Temperature, DO and pH were measured in every test vessel at test initiation and every 24 hours thereafter. Temperature was also measured continuously in the negative control. Hardness, alkalinity and conductance were measured at test initiation.	Specific Conductance= 320 µmhos/cm Alkalinity= 180 mg/L as CaCO ₃ <hr/> <u>Hardness:</u> EPA recommends 40 - 48 mg/L as CaCO ₃ (OECD recommends 10 - 250 mg/L) <u>pH:</u> EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8; (OECD recommends pH 6.0 - 8.5) <u>Dissolved Oxygen:</u> EPA recommends: Static: ≥ 60% during first 48 hrs and ≥ 40% during second 48 hrs; flow-through: ≥ 60%; (OECD guideline recommends at least 80% saturation value). <u>Temperature:</u> EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout). <u>Salinity:</u> EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰. Water quality should be measured at beginning of test and every 48 hours.
<u>Number of replicates/groups:</u> control: solvent control: treated ones:	1 N/A 1	<hr/> Recommended number of replicates include a control and five treatment levels with 2 replicates per level. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.
<u>Number of organisms per replicate /groups:</u> control: solvent control: treated ones:	10 N/A 10	<hr/> Number of organisms per replicate should be ≥ 10/concentration; OECD guideline recommends at least 7 fish/concentration.

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Parameter	Details	Remarks
		Criteria
Biomass loading rate	0.02 g fish/L	<i>Recommended static conditions are #0.8 g/L at #17EC and #0.5 g/L at > 17EC. Recommended flow-through conditions are #1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.</i>
<u>Test concentrations:</u> nominal: measured:	0 (negative control), 7.5, 15, 30, 60 and 120 mg ai/L <0.0143 (<LOD; control), 7.5, 15, 29, 60 and 120 mg ai/L	
Solvent (type, percentage, if used)	N/A; a solvent was not used	<i>The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.</i>
Lighting	16L:8D with a 30 minute transition period of low-light intensity	Light intensity at test initiation was 120 lux at the surface of the water of one representative test chamber. <i>The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12-16 hours.</i>
Feeding	Fish were not fed during the study	<i>Fish should not feed during the study.</i>
<u>Recovery of chemical</u> Frequency of determination Level of quantization Level of detection	0 and 96 hours 4.00 mg ai/L 0.0143 mg ai/L	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None reported	

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2. Observations:

Table 2: Observations

Parameter	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	-mortality -sub-lethal effects	
Observation intervals	Observations were made after 4, 24, 48, 72 and 96 hours of exposure	Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes	
Other observations, if any	None reported	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortalities occurred in the control or treatment groups resulting in NOAEC and LC₅₀ values of 120 and >120 mg ai/L, respectively.

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Table 3: Effect of DPX-MAT28 Technical on Mortality of *Lepomis macrochirus*.

Mean-Measured and (Nominal) Concentrations mg ai/L	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 3		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	10	0	0	0	0	0	0
7.5 (7.5)	10	0	0	0	0	0	0
15 (15)	10	0	0	0	0	0	0
29 (30)	10	0	0	0	0	0	0
60 (60)	10	0	0	0	0	0	0
120 (120)	10	0	0	0	0	0	0
NOAEC	120 mg ai/L						
LC ₅₀	>120 mg ai/L						
Positive control, if used mortality: LC ₅₀ :	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A- Not Applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

All control and treatment fish appeared normal and healthy throughout the definitive exposure period.

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Table 4: Sub-lethal Effect of DPX-MAT28 Technical on *Lepomis macrochirus*.

Mean-Measured and (Nominal) Concentrations mg ai/L	Observation Period		
	Endpoints- Day 1	Endpoints- Day 3	Endpoints- Day 4
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
7.5 (7.5)	A.N.	A.N.	A.N.
15 (15)	A.N.	A.N.	A.N.
29 (30)	A.N.	A.N.	A.N.
60 (60)	A.N.	A.N.	A.N.
120 (120)	A.N.	A.N.	A.N.
NOAEC	120 mg ai/L		
LOAEC	>120 mg ai/L		
EC ₅₀	>120 mg ai/L		
Positive control, if used % sublethal effect: EC ₅₀ :	N/A	N/A	N/A

A.N.- All fish appeared normal and healthy

N/A- Not applicable

C. REPORTED STATISTICS:

All toxicity values were visually determined due to a lack of mortality.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The complete lack of sub-lethal effects and mortality precluded the statistical analysis of the data. Therefore, all toxicity values were visually determined based on the mean-measured concentrations.

LC₅₀: >120 mg ai/L 95% C.I.: N/A

NOAEC: 120 mg ai/L

Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were identical to those of the study authors.

The results from the periodic screening analysis of the dilution water indicated the presence of the following elements: calcium (34.9 mg/L), chloride (4.8 mg/L), fluoride (1.2 mg/L), magnesium (12.8 mg/L), potassium (7.55

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mg/L) and sodium (19 mg/L).

The in-life portion of the definitive toxicity test was conducted from August 10 to August 14, 2007.

G. CONCLUSIONS:

This study is scientifically sound and classified as acceptable. The NOAEC and LC₅₀ values were 120 and >120 mg ai/L, respectively. Based on the results of this study, DPX-MAT28 Technical would be classified as practically non-toxic to *Lepomis macrochirus* in accordance with the classification system of the U.S. EPA.

LC₅₀: >120 mg ai/L 95% C.I.: N/A

NOAEC: 120 mg ai/L

Probit Slope: N/A 95% C.I.: N/A

III. REFERENCES:

Organization for Economic Cooperation and Development. 1993. Guideline 203: *Fish, Acute Toxicity Test*. OECD Guideline for Testing of Chemicals. Updated Guideline adopted on 17 July 1992.

U.S. Environmental Protection Agency. 1996. OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine*. Series 850- Ecological Effects Test Guidelines (*draft*).

APHA, AWWA, WPCF. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition. American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

